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Intestinal microbiota and its host: harmony or discord?

Next-generation probiotics to prevent and treat inflammatory bowel disease

Professor Philippe LANGELLA PhD
Directeur de Recherche, Responsable de l'équipe des Interactions des Bactéries Commensales et Probiotiques avec l'Hôte
(Institut MICALIS, INRA, Jouy-en-Josas)

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INTRODUCTION

There is no longer a need for verification of the importance of gut microbiota in human health, the major role of this organ is now well established. It appears particularly active in inflammatory bowel disease (IBD).

In 2008, *Faecalibacterium prausnitzii* (*F. prausnitzii*) was the first commensal anti-inflammatory bacterium identified on the basis of human clinical data (comparison of microbiota between IBD patients in remission and in relapse). Since this discovery, many studies have examined the correlations between *F. prausnitzii* and human intestinal dysbiotic diseases. This abundant and ubiquitous bacteria of our gut microbiota could, in the near future, play a major role in the new preventive and therapeutic strategies for gastrointestinal diseases. Thus, *F. prausnitzii* has great potential as a next generation probiotic for IBD patients.

THE INTESTINAL MICROBIOTA AND HUMAN HEALTH

Two major phyla have been identified in the gut microbiota[1-3]: Firmicutes and Bacteroidetes that are respectively Gram+ and Gram- bacteria. These two phyla constitute 80% to 90% of the microbiota. Among the bacteria of the Firmicutes phylum, *F. prausnitzii* is the major representative of the *Clostridium leptum* cluster IV.

Dysbiosis of the microbiota is often associated with immunological dysregulation. Eubiosis corresponds to a balanced distribution between the symbionts, regulators, and the pathobionts, proinflammatory pathogens. A preponderance of pathobionts signals a dysbiosis, which is an immune imbalance associated with the onset of an inflammatory process. A review by Round et al[4] investigating the influence of gut bacterial colonization on the development of the adaptive immune system confirms that disturbances of the bacterial microbiota cause dysregulation of adaptive immune cells. The authors consider this process to be at the origin of many non-infectious human diseases, in particular IBD.[4]

As early as 2004, a study reported a dysbiosis associated with antibiotic-associated diarrhea.[5] Many other studies have also reported an association between dysbiosis and Crohn's Disease,[6-11] and between dysbiosis and ulcerative colitis.[8,12,13] Similarly, Rajilic-Stojanovic et al showed that patients with irritable bowel syndrome (IBS) had dysbiosis of the gut microbiota.[14]

The microbiota has different functions in human health: a barrier function, metabolic and immune functions, and protection against pathogens. Notably, it has a role in the gut/brain axis. The gut microbiota appears to be a major player in many diseases (colorectal cancer, osteoarthritis, autism, diabetes, IBS, IBD, and so on). Such observations have led to the suggestion that microbiota and dysbiosis could be used as a potential source of next-generation probiotic bacteria.[15] These would be selected rationally on the basis of human clinical data, as was the case with the identification of *F. prausnitzii*.

Traditional probiotics (*Bifidobacterium, Lactobacillus, Saccharomyces,* and *Escherichia coli*) have been used for a long time. They are provided via foods and dietary supplements. Next-generation probiotics, often referred to as "live biotherapeutics" in the literature, are microorganism-drugs; the prototypes are *Akkermansia, Bacteroides* and *Faecalibacterium*.[16] Food lactic acid bacteria are also used in some laboratories as bacteria-drugs; they are genetically engineered to produce and deliver proteins of interest in human health.

FAECALIBACTERIUM PRAUSNITZII

Description of *F. prausnitzii*

F. prausnitzii, a Gram+ bacillus, is a member of the phylogenetic core as demonstrated in the MetaHit study.[17] In this study, it was present in the group of 18 bacteria identified in 120 healthy volunteers whose microbiota was analyzed.[17] It is the most abundant bacterium in the gut microbiota (3.5% to 5% of commensal bacteria), regardless of the region of the world where the analysis is made. *F. prausnitzii* is also a major producer of butyrate, a short chain fatty acid. However, this bacterium is extremely sensitive to oxygen, which makes it difficult to grow and handle. The necessity of using an anaerobic chamber makes its production technically difficult, requiring great expertise.

The history of *F. prausnitzii* identification

The role of *F. prausnitzii* was discovered during a collaborative program between the INRA (Institut National de Recherche Agronomique) and Saint-Antoine hospital in Paris. The study included 20 patients with ileal Crohn's disease undergoing ileocecal resection. Prior to resection, two groups of patients were identified: the first group with a normal amount of *F. prausnitzii* and the second showing decreased levels of *F. prausnitzii*. Six months post-surgery, colonoscopy revealed a group in remission and a group experiencing disease relapse. Patients in remission were those with a normal *F. prausnitzii* population whereas patients with Crohn's disease recurrence had insufficient numbers.

On this basis, a study was carried out in a model of TNBS-induced colitis (trinitrobenzene sulfonic acid), in order to seek correlations between IBD recurrence and reduction of *F. prausnitzii*, to provide evidence of the potential protective and anti-inflammatory effects of *F. prausnitzii*.[8] The results show that in this model both the bacterium and the supernatant have protective effects related to the decrease of proinflammatory cytokines and the increase in the production of anti-inflammatory cytokines.[8] This work, published ten years ago in PNAS, allowed for the identification of the "good guys" (symbionts) and "bad guys" (pathobionts) as actors within our microbiota. This study, regarded as pioneering, is today still considered a reference in our knowledge about the microbiota, the related publication being one of the ten most cited articles in this area.

New strategy based on dysbiosis to select next-generation probiotics

A new strategy for the isolation of probiotic bacteria was introduced as a result of this study's observations. The standard procedure for isolating new probiotic strains is based on a panel of 50 to 100 bacterial candidates whose potential health effects are assessed in cell or animal models. This procedure is likely to reveal 1 to 3 new probiotic bacteria. The starting point of the proposed strategy is based on the observation of a dysbiosis identified by comparing the microbiota of healthy subjects or patients in remission of relapsing patients, allowing for the identification of the missing bacteria in relapsing subjects and the analysis of their properties. We carried out this isolation procedure in order to identify *F. prausnitzii*, the beneficial properties of which were then validated in cellular or animal models.

IMPLICATION OF *F. PRAUSNITZII* IN DYSBIOSIS

In addition to this pioneering study, various studies have shown that *F. prausnitzii* is reduced in patients with IBD.[9,18,19] We can therefore consider today that *F. prausnitzii* is a kind of biomarker of intestinal health, not only because it has been identified in Crohn's disease and ulcerative colitis, but because it appears also to be reduced in patients with colorectal cancer and in patients with IBS.[9,18,19]

One study also identified a clear correlation between the count of *F. prausnitzii* and the risk of relapse in patients treated with Infliximab for Crohn's disease.[20] At the time of Infliximab treatment discontinuation, the level of *F. prausnitzii* varied from one subject to another. The survival curves showed that the higher the level, the less the patients relapsed, showing that *F. prausnitzii* is predictive of relapse in patients with Crohn's disease treated with Infliximab.[20]

Animal models of stress and colitis

The model used in the study by Sokol et al[8] was a classic murine model in which a single challenge was performed with TNBS to induce colitis in the model reproducing a flare in a patient with IBD.

More recently, a chronic model has been used, which better represents the chronicity and succession of flares in Crohn's disease.[21] In this model we observed that both *F. prausnitzii* and its supernatant had positive effects, *F. prausnitzii* showing a protective role on the epithelium in chronic colitis induced by DNBS (dinitrobenzene sulphonic acid).[21]

The possible impact of *F. prausnitzii* on abdominal pain has been studied in a murine model of neo-maternal separation within 10 days after birth, inducing visceral hypersensitivity.[22] Compared with mice not separated from the mother, mice in the neo-maternal separation group showed a significant increase in visceral hypersensitivity. Treatment with *F. prausnitzii* in these models, evaluated in terms of visceral-motor response to treatment, revealed a significant anti-nociceptive effect on hypersensitivity. The intestinal permeability that had been increased by maternal separation stress, was restored by the *F. prausnitzii* treatment, showing its ability to strengthen the epithelial barrier of the gut.[22]

The modes of action of *F. prausnitzii*

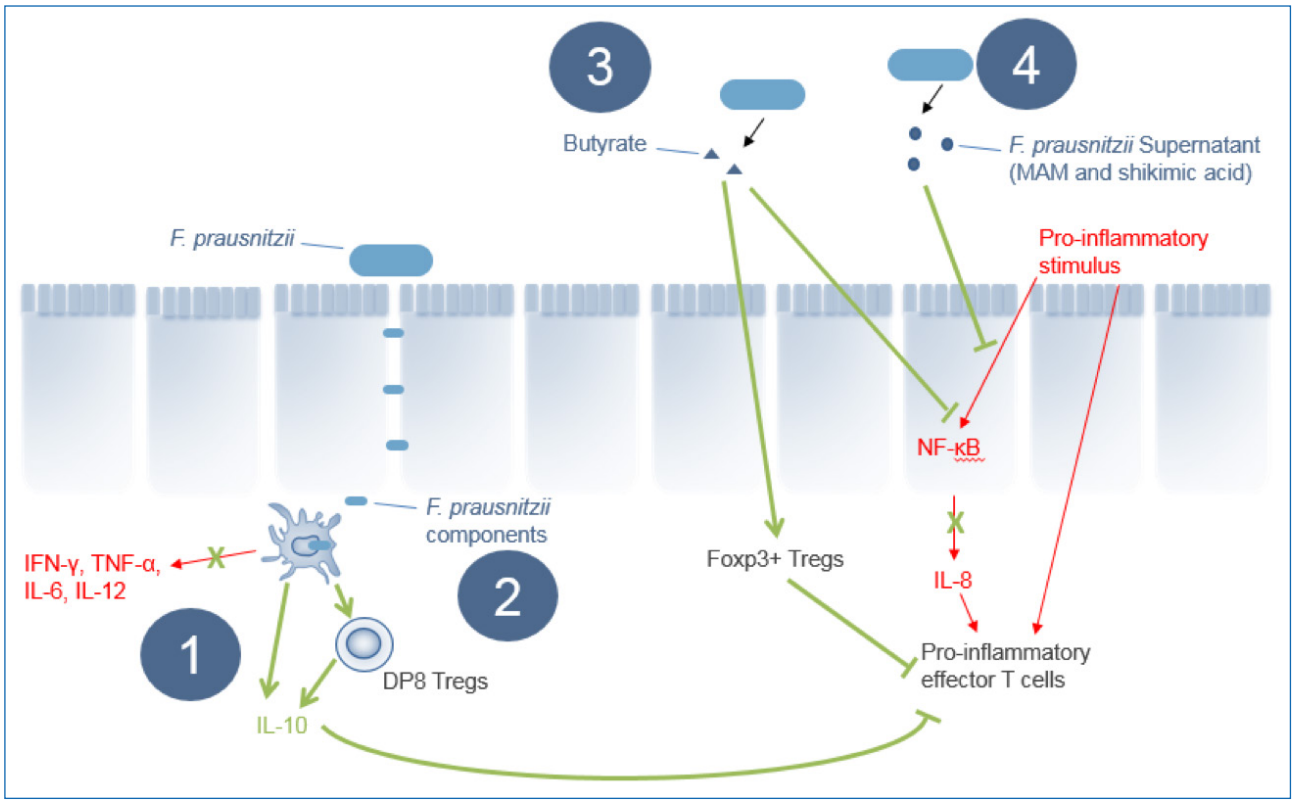
Various analyses have been performed to determine the mode of action of *F. prausnitzii*.[8,23-26]

At first, seven peptides were identified by mass spectrometry of the supernatant. A metabolomic analysis in a simplified system was then used in vitro to identify metabolites that may have an effect on *F. prausnitzii* mechanisms of action. In vivo, in axenic mice, *E. coli* was used as a companion to allow the implantation of *F. prausnitzii*. Induction of colitis by TNBS showed protection against colitis in mice harboring *E. coli* and *F. prausnitzii*. A simple metabolomic analysis revealed, in protected mice, metabolites such as raffinose, shikimic acid and salicylic acid,[23] two acids used for their anti-nociceptive and anti-inflammatory effects.

The supernatant analysis in this study also identified seven peptides, all derived from a MAM protein (microbiota anti-inflammatory molecule), which have a key role in the anti-inflammatory modes of action of *F. prausnitzii*. The use of lactococci delivering MAM cDNA showed positive impacts in a model of DNBS-induced colitis.[24]

The different mechanisms of action identified by studies conducted for this purpose are presented in Figure 1.[8,23-26]

Figure 1. *Faecalibacterium prausnitzii*, summary of the various mechanisms of action that have been published.[8,23-26]



New strains of *F. prausnitzii* have been discovered recently. The selection of the strain CNCM-4573[27] and the sequencing of the genome of these bacteria[28] has allowed for a better knowledge of these new strains of *F. prausnitzii*.

HOW TO USE *F. PRAUSNITZII* AFTER 10 YEARS OF RESEARCH ON THE IDENTIFICATION OF ITS PROPERTIES?

Given the identification of *F. prausnitzii* and its effects, it could be interesting to use prebiotics and probiotics to increase its population. It may also be considered to utilize it alone either as a drug or as what is referred to as a "novel food" (see European legislation). Finally, it can be used to produce post-biotics such as MAM protein or other molecules secreted by *F. prausnitzii*, and to use them as drugs.

Based on preclinical models obtained in mice, the proof of concept in humans has successfully passed through the following three steps: **1)** Production of *F. prausnitzii* as a biologically active lyophilisate produced in accordance with Good Manufacturing Practices; **2)** Identification of its mode of action; and **3)** Demonstration of its lack of toxicity.

As a next-generation probiotic, *F. prausnitzii* will be undergoing, within two years, clinical trials in patients with IBD; these studies will be carried out by Exeliom Biosciences, a start-up company created by INRA in November 2016. The objective is to make available freeze-dried capsules of *F. prausnitzii* that could be given to patients with IBD.

Lactobacilli producing AhR agonists, other next-generation probiotics

Recent work on Card9 mice (Crohn's disease susceptibility gene) has identified new strains of probiotic bacteria. A colitis induction study was conducted in Card9 KO mice with the aim of identifying new generation probiotic bacteria[29] In this type of mouse, the metabolism of tryptophan is deficient. The study showed that the addition of a trio of lactobacilli producing tryptophan metabolites helped to protect the mouse from the

effects of induced colitis, an effect explained by an increase in AhR activity and the secretion of IL-22.[29] These results suggest that AhR agonist-producing lactobacilli could be used as next-generation probiotics alone or in combination with other new generation probiotics.

COMMENTS, CONCLUSIONS

Many studies have now established that genes can shape the gut microbiota and its function, strongly impacting human health. In IBD, the function of the intestinal microbiota is impaired, but this dysbiosis seems to be reduced by new generation probiotic bacteria derived from the microbiota.

Today, future prospects for research are the further characterization of promising next-generation probiotics and their mode of action, comparison of their effects in single strains or consortia of these strains, and the initiation of clinical trials in humans aiming at achieving a proof of concept for the use of these next-generation probiotics in IBD patients.

REFERENCES (Underlined references link to PubMed abstracts)

1. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 2007; 449(7164):811-818.
2. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI. Evolution of mammals and their gut microbes. Science 2008; 320(5883):1647-1651.
3. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, Ugarte E, Muñoz-Tamayo R, Paslier DL, Nalin R, Dore J, Leclerc M. Towards the human intestinal microbiota phylogenetic core. Environ Microbiol 2009; 11(10):2574-2584.
4. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009; 9(5):313-323.
5. Beaugerie L, Petit JC. Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. Best Pract Res Clin Gastroenterol 2004; 18(2):337-352.
6. Seksik P, Rigottier-Gois L, Gramet G, Sutren M, Pochart P, Marteau P, Jian R, Doré J. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. Gut 2003; 52(2):237-242.
7. Sokol H, Seksik P, Rigottier-Gois L, Lay C, Lepage P, Podglajen I, Marteau P, Doré J. Specificities of the fecal microbiota in inflammatory bowel disease. Inflamm Bowel Dis 2006; 12(2):106-111.
8. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn's disease patients. PNAS 2008; 105 (43):16731-16736.
9. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis 2009; 15(8):1183-1189.
10. Dicksved J, Halfvarson J, Rosenquist M, Järnerot G, Tysk C, Apajalahti J, Engstrand L, Jansson JK. Molecular analysis of the gut microbiota of identical twins with Crohn's disease. ISME J 2008; 2(7):716-727.
11. Willing B, Halfvarson J, Dicksved J, Rosenquist M, Järnerot G, Engstrand L, Tysk C, Jansson JK. Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm Bowel Dis 2009; 15(5):653-660.
12. Martinez C, Antolin M, Santos J, Torrejon A, Casellas F, Borruel N, Guarner F, Malagelada JR. Unstable composition of the fecal microbiota in ulcerative colitis during clinical remission. Am J Gastroenterol 2008; 103(3):643-648.
13. Varela E, Manichanh C, Gallart M, Torrejón A, Borruel N, Casellas F, Guarner F, Antolin M. Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Aliment Pharmacol Ther 2013; 38(2):151-161.
14. Rajilić-Stojanović M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, de Vos WM. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 2011; 141(5):1792-1801.
15. Patel R, DuPont HL. New approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clin Infect Dis 2015; 60 Suppl 2:S108-121.
16. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat Microbiol 2017; 2:17057.

17. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al; MetaHIT Consortium. Enterotypes of the human gut microbiome. Nature 2011; 473(7346):174-180.
18. Willing BP, Dicksved J, Halfvarson J, Andersson AF, Lucio M, Zheng Z, Järnerot G, Tysk C, Jansson JK, Engstrand L. A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. Gastroenterology 2010; 139(6):1844-1854.
19. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 2012; 13(9):R79.
20. Rajca S, Grondin V, Louis E, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, et al. Alterations in the intestinal microbiome (dysbiosis) as a predictor of relapse after infliximab withdrawal in Crohn's disease. Inflamm Bowel Dis 2014; 20(6):978-986.
21. Martín R, Chain F, Miquel S, Lu J, Gratadoux JJ, Sokol H, Verdu EF, Bercik P, Bermúdez-Humarán LG, Langella P. The commensal bacterium Faecalibacterium prausnitzii is protective in DNBS-induced chronic moderate and severe colitis models. Inflamm Bowel Dis 2014; 20(3):417-430.
22. Miquel S, Martín R, Lashermes A, Gillet M, Meleine M, Gelot A, Eschalier A, Ardid D, Bermúdez-Humarán LG, Sokol H, Thomas M, Theodorou V, Langella P, Carvalho FA. Anti-nociceptive effect of Faecalibacterium prausnitzii in non-inflammatory IBS-like models. Sci Rep 2016; 6:19399.
23. Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn's disease. Gut 2016; 65(3):415-425.
24. Breyner NM, Michon C, de Sousa CS, Vilas Boas PB, Chain F, Azevedo VA, Langella P, Chatel JM. Microbial anti-inflammatory molecule (MAM) from Faecalibacterium prausnitzii shows a protective effect on DNBS and DSS-induced colitis model in mice through inhibition of NF-κB pathway. Front Microbiol 2017; 8:114.
25. Miquel S, Leclerc M, Martin R, Chain F, Lenoir M, Raguideau S, Hudault S, Bridonneau C, Northen T, Bowen B, Bermúdez-Humarán LG, Sokol H, Thomas M, Langella P. Identification of metabolic signatures linked to anti-inflammatory effects of Faecalibacterium prausnitzii. MBio 2015; 6(2). pii: e00300-15.
26. Sarrabayrouse G, Bossard C, Chauvin JM, Jarry A, Meurette G, Quévrain E, et al. CD4CD8αα lymphocytes, a novel human regulatory T cell subset induced by colonic bacteria and deficient in patients with inflammatory bowel disease. PLoS Biol 2014; 12(4):e1001833.
27. Martín R, Miquel S, Benevides L, et al. Functional characterization of novel Faecalibacterium prausnitzii strains isolated from healthy volunteers: a step forward in the use of F. prausnitzii as a next-generation probiotic. Front Microbiol 2017; 8:1226.
28. Benevides L, Burman S, Martin R, et al. New insights into the diversity of the genus Faecalibacterium. Front Microbiol 2017; 8:1790.
29. Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nat Med 2016; 22(6):598-605.